# GRID FRAP PATTERNING REVEALS A DISPERSIVE EFFECT IN THE BULK OF A LINEARLY CONTRACTING MICROTUBULE NETWORK



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#### 3.1 Abstract

Deliberate photobleaching has provided a means for quantitatively identifying the diffusive, reactive, and convective effects of fluorescent molecules in biological systems, but have been sparsely applied to active systems. We photobleach a grid pattern onto a highly-connected fluorescent microtubule network to observe the reorganization of the filaments in the bulk of the network as they contract to form an aster. Analysis of the fluorescent squares shows that while there is a linear contraction of the microtubules toward the center of the network, the microtubules that make up the fluorescent squares also exhibit a local dispersive effect. Use of different motor speeds and an advection-diffusion equation where diffusion is treated as the null hypothesis reveals that the dispersion comes largely due to microtubules sliding relative to each other from the motors processing along them.

#### 3.2 Introduction

From the population level where animals move as a collective to the subcellular with the formation of such structures as the mitotic spindle, coordinated movement and emergent patterning can be observed across all biological scales. The question of how such self-organization emerges has led to growing theoretical [1–4] and experimental efforts over the past quarter of a century. While the experimental system of choice varies from the granular particles [5, 6] to the cellular [7, 8], in vitro active matter systems using elements of the cytoskeleton offer a powerful means to study the diverse array of structures found in biology [9]. By mixing multimerized motors with filaments, a broad range of ordered patterns emerge [10, 11]. Such systems have become increasingly sophisticated of late with the ability to spatially select where motors couple and drive filament redistribution and thus affect local ordering [12–14]. A common observation from these assays is that the geometry of the light-activating region that induces such selective network formation influences the behavior of the network to produce local order. However, the filament rearrangement that occurs in the bulk of these activated networks is not well known. Recent efforts in the last quarter century have led to several models that quantitatively describe the redistribution of microtubules due to the interactions with motors [15–19]. However, these quantitative descriptions have not been coupled with experimental evidence to reveal the extent of the reorganization in the bulk of the network.

One of the most important tools in microscopy for the imaging of biological systems is the ability to fluorescently label different molecules and image them. This is especially powerful when imaging multiple distinct molecules and using different wavelength-dependent filters to independently measure their position with the same sample. While it is common to avoid exposing fluorescent particles to too much light and permanently turn off their fluorescence, a phenomenon called photobleaching, this can also become a powerful tool. Fig. 3.1 shows this benefit in more detail. A laser can be used to shine an intense beam of light that photobleaches molecules in a select region, leaving the molecules outside of the beam path to remain fluorescent and unaffected (Fig. 3.1B). After some amount of time, the fluorescent molecules can begin to



Figure 3.1: Concept of fluorescence recovery after photobleaching (FRAP). (A) A laser shines on a sample of red fluorescent molecules in order to bleach the molecules within its path, (B) leaving behind a circular pattern within which all of the particles are dim and outside of which the molecules are still fluorescent. (C) After some amount of time, the fluorescent molecules will fill in the bleached region, leading to a recovery of fluorescence in the area.

populate the bleached region again, leading to recovery of fluorescence (Fig. 3.1C). This technique is known as fluorescence recovery after photobleaching (FRAP).

FRAP has been applied extensively on a range of *in vitro* and *in vivo* systems to examine the movement of fluorescent particles that enter the darkened region [20–24]. These fluorescence recovery studies have typically been tied to various extensions of diffusion equations, whether they further involve convective flow, reaction of molecules, or transport [16, 21–23, 25]. Recent work has demonstrated the effectiveness of FRAP assays in *in vitro* active matter systems, but have been specifically applied to systems of high filament densities [26–28]. Even so, use of photobleaching to examine movement of the key players in active systems has still been applied sparingly in the field and are less common in systems where the filament network contracts to organize over a smaller volume and increase density by orders of magnitude. In such cases of network compaction, bleaching regions within the bulk of the network can

similarly aid in determining the length scale over which filaments are redistributing or elastically contracting.

In the work presented here, we incorporate photobleaching into the lightactivated motor dimerization system [13] and examine movement of microtubules in the bulk of the network during the contraction stage on the way to forming an aster. By photobleaching the microtubule network with a grid pattern so as to observe both angular and radial dependencies, we reveal a local dispersal of microtubules amidst a global contraction of the filament network. Use of different motors shows that motor speed changes the contraction rate but maintains the size of this dispersal effect. We further examine this phenomenon using finite element method (FEM) to numerically solve an advection-diffusion model on our photobleach data to validate that the dispersal effect can occur merely due to passive diffusion and that the dispersal is likely generated by the sliding of filaments from the crosslinking motors.

#### 3.3 Results

#### 3.3.1 Photobleaching a grid pattern

In addition to the projector required to induce dimerization of kinesin through the iLid-micro system [29] and image the microtubules, we include a laser for photobleaching with a peak intensity of  $\approx 645$  nm and in whose beam path we include a cylindrical lens array mounted to a motorized rotation mount to produce parallel lines of light. A gimbal-mounted mirror allows us to sweep the parallel lines to create thicker lines while the rotation mount allows us to rotate the lens array by 90° before sweeping parallel lines in the orthogonal direction to generate the grid pattern. Further details of the custom microscope build are available in the Materials and methods section and in Sec. B.1.6 of Appendix B.

Fig. 3.2 shows three examples of the grid photobleaching pattern onto a microtubule network at different time points in its life history and the subsequent deformations of the bleached lines and fluorescent squares. These three instances roughly correspond to (A) the initial stages of contraction where the coupled microtubules begin to pull away from the microtubules that fall outside of the activated light region (outlined with a beige dashed line in the pre-photobleaching frame); (B) in the middle of contraction at least two minutes after the network has contracted away from the reservoir of uncoupled



Figure 3.2: Photobleaching a grid pattern onto the contracting microtubule network. Here, three different time points during microtubule network contraction are shown where photobleaching is applied: (A) early in contraction as the network pulls away from the reservoir of uncoupled microtubules; (B) in the middle of the contracting phase; and (C) toward the end of contraction before the network no longer appears to shrink. In addition to the image of the network before photobleaching (leftmost column) and the image taken immediately after photobleaching (0 seconds), images of the network 60 seconds, 120 seconds, and 200 seconds after photobleaching are also shown. Dashed line in the pre-bleached early contracting microtubule network outlines the circular activation pattern used to dimerize iLid- and micro-tagged motors to each other. Scale bars in the t = 200 sec column apply to their respective rows of images.

filaments; and (C) toward the end of contraction when the size of the network no longer appears to decrease. As the second column in Fig. 3.2 shows, upon photobleaching the grid pattern, individual fluorescent squares, which we will call unit cells, are produced. Over a minute after photobleaching (middle column), unit cells contract toward the center of the network while the photobleached lines appear to thin. By two minutes after photobleaching, neighboring unit cells appear to blend into each other and at later times any remnants of the photobleached pattern disappear.

# 3.3.2 Tracking of fluorescent squares shows global contraction but local dispersion

To better understand the global network contraction toward the center and the local spreading of fluorescent filaments before filaments of neighboring unit cells begin to interact, we segmented individual unit cells and measured their centroids and areas over successive frames while preserving total fluorescence intensity. This image processing is detailed more closely in Section B.2 of Appendix B. Fig. 3.3A shows that individual unit cells in each experiment are assigned an identification number as labeled at the upper left of each unit cell. Fig. 3.3B shows line plots of the distance of each unit cell centroid as labeled in Fig. 3.3A from the center of the network over successive frames. We see that while each centroid moves roughly linearly in time toward the origin, those centroids further away from the origin appear to move toward the origin faster than those closer to the network center. We computed the slope of the distance-to-time relationship of each unit cell across all of the experiments and compiled them in Fig. 3.3C to find that the contraction speed linearly increases with distance from the center, indicating a general linear contraction of the microtubule network. Upon fitting a line through the origin, we find that the contraction rate  $\alpha = 1.6 \times 10^{-3} \text{ s}^{-1}$  with the 95% credible region falling between  $1.4 \times 10^{-3} \text{ s}^{-1}$  and  $1.8 \times 10^{-3} \text{ s}^{-1}$ .

Despite the linear global contraction observed for the centroids, a more macroscopic examination of the unit cells does not show a pure contraction of their constitutive microtubules. In fact, instead of each unit cell contracting into smaller squares, which would be expected for a strictly contracting network subject to the linear contraction speed scaling computed before, we observe a dispersion of the filaments can be seen in successive frames of the photobleached network after contraction. Fig. 3.3D shows the normalized area of each unit cell as a function of time in gray while the mean normalized area is shown in blue. A purely contracting field subject to the linear contraction rate as measured by tracking the movement of the centroids would mean that the normalized areas would fall on the dashed red line, which scales as  $(1 - \alpha t)^2$ (see Sec. B.5 of Appendix B), yet each unit cell maintains an area greater than the pure contraction bound. In fact, on average, the unit cells appear to conserve their area. This area-preserving effect illustrates that despite the global contraction of the network, filaments are locally spreading counter to this contraction and reorganizing in the bulk.



Figure 3.3: Incorporation of photobleaching into a contracting microtubule network. (A) Image of the microtubule network after photobleaching with a grid pattern. Numbers in the upper left of each unit cell denote an identification tag for tracking. Data for unit cells toward the outer edges of the network are truncated when cells appear to merge with each other. (B) Centroids of each unit cell are tracked for up to 90 seconds after photobleaching and plotted as distance from the center of the network against time. Numbers to the left of each t = 0 sec point denote the unit cell ID as indicated in (A). (C) Plot of unit cell contraction speed as a function of their average distances from the center of the network. The contraction speed of each unit cell is obtained by fitting the distance vs time data found in (B) to a line. The median contraction rate is  $\alpha = 1.6 \times 10^{-3} \text{ s}^{-1}$ . (D) The area of each unit cell is normalized against their initial area as obtained by the unit cell segmentation scheme and plotted as a function of time. The mean normalized area is plotted in blue among individual unit cells (gray). Dashed red line corresponds with the normalized area if the unit cells purely contracted according to the median contraction rate obtained in (C). Number of unit cells decrease at later times as they begin to merge with neighboring cells.



Figure 3.4: Contraction rate and normalized area of Ncd281. (A) Comparison of contraction rates of the microtubule network between Ncd236 and Ncd281. Error bars denote range of 95% credible region (B) Normalized area of unit cells over time. Red dashed line denotes expected trajectory of unit cell areas subject purely to contraction rate. Normalized areas of individual unit cells noted in grey with their averages at each time point after photobleaching noted in blue.

#### 3.3.3 Dispersion of microtubules is unaffected by motor speed

The unchanging unit cell areas suggest two possible dispersive effects. One possibility is that some microtubules in the bulk may be disconnected from the network and thus allowed to diffuse until recaptured by a bound motor. If diffusion plays a clear role, then we hypothesize that decreasing the contraction rate would cause the normalized areas to become larger in time while increasing the contraction would drive unit cells to contract faster than diffusion can disperse the microtubules, thus causing unit cell areas to decrease in time. Another possibility is that the motors are driving large reorientation and repositioning of microtubules in the network rather than locking them in relative to their coupled filaments. Such an effect would lead some microtubules to extend outward from the center of unit cells and disperse, particularly if the network is not locally ordered. To test whether motors play a role in the dispersion, we applied the same photobleaching experiments using a variant of the Ncd236 used that is slower without a notable change in ATP hydrolysis rate. This Ncd variant, Ncd281, was shown to be roughly two-thirds the speed of Ncd236 and is otherwise assumed to have roughly the same kinetic properties [30].

Fig. 3.4A shows that upon using the slower Ncd281, the contraction rate of the microtubule network decreases to  $1.4 \times 10^{-3}$  s<sup>-1</sup> with a 95% credible region between  $1.2 \times 10^{-3}$  s<sup>-1</sup> and  $1.5 \times 10^{-3}$  s<sup>-1</sup>. When examining the normalized area of the unit cells generated from the photobleach pattern, unit cells on average remain roughly the same area in the network, decreasing on average to only 0.97 of its original size. Had the dispersion been due to diffusion of the microtubules, the dispersive effect should have made unit cells expand faster than the global contraction would have shrunk them, making the unit cells grow larger than their initial area. However, as the unit cells maintain their size with the slower motor, this preservation of unit cell size is suggestive that the dispersive pattern comes largely due to the continual reorganization of microtubules by the motors. Indeed, we find that even while using faster motors, the unit cells are still roughly the same size. Photobleaching experiments using K401 expressed in bacteria and K401 expressed in insects, which have different motor speeds, show that unit areas remain well preserved.

## 3.3.4 A grid-like photobleach pattern simultaneously shows the effects of diffusion and advection

Due to the advection of the microtubules toward the center of the network and a seeming diffusive effect that causes the unit cells to disperse, we model the contraction process using an advection-diffusion model of the tubulin concentration c(r, t). At its most generic, such a model has a material flux **J** of the form

$$\mathbf{J} = -D\nabla c + \mathbf{v}(\mathbf{r})c,\tag{3.1}$$

where D is the diffusion constant and  $\mathbf{v}(\mathbf{r})$  is the velocity profile of the advective flow as a function of distance from the center of contraction r. Here, as motivated by results shown in Sec. 3.3.2, the velocity function is linear with r and negative in the radial direction:

$$\mathbf{v}(\mathbf{r}) = -\frac{v_{\rm m}}{R}\mathbf{r}.\tag{3.2}$$

Here, we elect to make the constant coefficient a fraction where  $v_{\rm m}$  the velocity at r = R. As a result, the advection-diffusion model takes the form of

$$\frac{\partial c}{\partial t} = D\nabla^2 c + \nabla \cdot \left[\frac{v_{\rm m}}{R}\mathbf{r}c\right]. \tag{3.3}$$

To better understand the behavior of the concentration profile subject to this advection-diffusion process, we derived a general solution when Eq. 3.3 is sub-

ject to a no-flux boundary condition to represent our experimental efforts to fully disconnect the coupled microtubules in the iLid-activation region from the reservoir of filaments outside the activation circle. This derivation is available



Figure 3.5: Concentration profile for a grid pattern initial condition at six time points. (A) Heatmap of the concentration profile in the circle. A line is traced at 0 degrees (purple), 15 degrees (blue), 30 degrees (red), and 45 degrees (green) from the origin to the outer radius of the defined geometry and in correspondence with (B-E), respectively. Later times in the line plots are denoted by increasingly lighter color tones. Here,  $D = 0.1 \frac{\mu m^2}{s}$ and  $v_m = 0.1 \frac{\mu m}{s}$ .





Figure 3.6: Comparison of advection-diffusion FEM solution to experimental results. Comparison of experimental image (top row), FEM solution (middle row), and Line profiles of relative tubulin concentration at (A) t = 0 sec, (B) t = 40 sec, (C) t = 80sec, (D) t = 120 sec, and (E) t = 160 sec. Concentration profiles are from concentration along blue line shown in the t = 0 image. Red 'X' in t = 0 concentration profile denotes the FEM initial condition implemented in COMSOL. Red shaded regions are set by concentration profiles where  $1.8 \times 10^{-3} \text{ s}^{-1} \leq \alpha \leq 2 \times 10^{-3} \text{ s}^{-1}$  and  $0.05 \frac{\mu m^2}{s} \leq D \leq 0.15 \frac{\mu m^2}{s}$ . COMSOL simulated concentration heatmaps are generated using  $D = 0.15 \frac{\mu m^2}{s}$  and  $\alpha = 1.9 \times 10^{-3} \text{ s}^{-1}$ .

in Sections B.10 and B.12 of Appendix B for the 1D and 2D cases, respectively. We further derive three specific solutions each with different initial conditions: (1) a uniform concentration; (2) a uniform concentration but with tubulin removed in a smaller region  $r < R_0$  as if performing a circular FRAP assay; and (3) a Gaussian concentration profile with a circular FRAP pattern. This deeper exploration into the model allowed us to determine that the concentration tends toward a Gaussian profile with  $\sigma^2 = \frac{DR}{v_m}$ . These three initial conditions also suggest that the role of D and  $v_m$  would be difficult to distinguish with a circular FRAP pattern as the removal of molecules in the inner region produces a concentration gradient that drives diffusion in the same direction as advection, further validating the choice of using a grid pattern in the experiment.

As a result of the agreement found between numerical solutions obtained by finite element methods (FEM) using COMSOL Multiphysics® and the analytical solutions under each of the three initial conditions, we turn fully to the

use of FEM simulations on Eq. 3.3 for the grid photobleaching pattern. We start with the initial condition of a gridlike pattern where the concentration in the gridlines is 0 and those in the individual unit cells are set to  $c_0$ . Individual unit cells have side lengths of 2.5 µm and have center-to-center distances of 5  $\mu$ m from each other. This initial condition is reflected in Fig. 3.5A, where the white squares denote initial concentrations of  $c_0$  while the surrounding black regions mark no molecules. We note that this initial condition has an angular dependence. By subjecting molecules to the same diffusion constant of  $D = 0.1 \frac{\mu m^2}{s}$  and maximum contraction velocity of  $v_m = 0.1 \frac{\mu m}{s}$ , we see that the concentration localized to the unit cells disperse faster than the advection pushes the materials toward the origin. However, at longer times such as at t = 200 seconds shown in Fig. 3.5A, we observe an accumulation of molecules toward the origin. Fig. 3.5B-E show line traces of the concentration at different angles from the origin to the boundary of the system in  $15^{\circ}$ increments (and as defined by the four lines in Fig. 3.5A). As each of the line traces illustrate, the concentration profiles spread out at a faster rate than the advective current flows materials toward the origin over the short times, but in the long time limit the concentration accumulates toward the center to generate the familiar Gaussian distribution. We explore the implications of tuning the advection and diffusion parameters as well as the derivation of the Péclet number that reveals this interplay in the Section B.15 of Appendix B

We next examine how well this advection-diffusion model matches that of the experimental data. To do this, rather than manually designing a simulation environment that mimics a typical photobleached MT network, we imported an image of a contracting network in the first frame after photobleaching the filament network and supplied it into COMSOL as our initial condition (implementation in COMSOL is available in Section B.13 of Appendix B). From there, we allowed the system to involve subject to our advection-diffusion model for a range of contraction rate  $\alpha \equiv \frac{v_m}{R}$  and diffusion constant D parameters. Fig. 3.6 shows a comparison of the advection-diffusion model to the experimental results at various time points using an imageset where the microtubule network is photobleached toward the end of contraction. Here, we can see that while the advection-diffusion model at first shows good agreement between experiments and numerical results, by about the 160 second mark the experimental results appear to show a more uniform concentration profile than the advection-diffusion results suggests it should have (Fig. 3.6).

An examination of concentration profiles further highlights the limits to the model. We drew a line along both the image and the FEM results, shown as the blue line in the image under Fig. 3.6A. We mark the range of concentration profiles at later timepoints as the red shaded region with a contraction rate  $\frac{v_{\rm m}}{R} = 1.8 \times 10^{-3} \text{ s}^{-1}$  and diffusion constant  $D = 0.15 \frac{\mu \text{m}^2}{\text{s}}$  setting one bound of the shaded region and  $\frac{v_{\rm m}}{R} = 2.0 \times 10^{-3} \text{ s}^{-1}$  and  $D = 0.05 \frac{\mu \text{m}^2}{\text{s}}$  setting the other bound. Here, we can see that the concentration profile of the experiments agrees well with the FEM solution over shorter time scales. At about 2 minutes (Fig. 3.6D), the concentration profile appears to show an increase in tubulin concentration in the local minimum located  $\approx 15 \ \mu m$  from the network center. By t = 160 sec, the advection-diffusion model overestimates the concentration of tubulin and does not recapitulate the flattening concentration profile found in the experiments. Thus, the advection-diffusion model, while able to capture the competing effects of the advection toward the network center and the dispersion that has been observed in experiments over shorter time scales begins to diverge from experimental results at later times. This finding further backs up the finding that passive diffusion alone cannot account for the dispersive effect found in the experiments. We further elaborate on the implications of the disagreement between model and observations in the Discussion section.

#### 3.4 Discussion

By imposing a grid photobleaching pattern onto fluorescent squares, we observe that despite a global contraction that scales linearly with the distance from the network center, the microtubules are constantly redistributing and interacting with new microtubules at the local level due largely to the motors moving filaments relative to one another. The dispersive effect observed during the contracting process of the network comes in contrast to other active contraction systems where an elastic contractile effect is the dominant effect [12, 19]. However, the work presented here differs in the choice of motors used (kinesin motors in this work as opposed to dynein for Foster *et al.* and myosin in Schuppler *et al.*) and in the case of Schuppler *et al.*, flexible actin filaments are heavily crosslinked by both active and inactive myosin motors and remain connected to the region outside of the activation zone.

Our results show that regardless of how much the microtubule network has already contracted and by extension independent of the density of microtubules from its initially uniform distribution to when it is nearing the end of contraction, the area that the fluorescent microtubules from the same unit cell occupy is on average time-independent. From using motors with different speeds and finding that the area of coverage by microtubules belonging to the same fluorescent unit cell is roughly preserved during the contraction process, the dispersion may come from coupled filaments sliding due to polarity sorting, as similarly observed in the dense microtubule limit [26]. We note that previous uses of photobleaching of a microtubule network revealed that the area of the bleached region remains the same, much like the area preservation of the fluorescent squares observed here, and suggested that the filaments did not slide much relative to each other [27]. In contrast, due to the global contraction of the filament network, for the unit cells to maintain their area of fluorescence rather than contract by the contraction rate of the system suggests that the filaments must be constantly exchange with their nearest neighbors throughout the global contraction, leading to a local expansion of individual filaments and thus preserving the area that these fluorescent microtubules sweep out. While work by Tayar *et al.* is performed in the high density limit and thus leads to alignment of filaments locally, our work and the seemingly isotropic dispersion of filaments suggests lower alignment among filaments [27].

When we used the advection-diffusion model (Eq. 3.3) as our null hypothesis and implemented our experimental data as initial conditions, we found that even when setting a range of parameters for the contraction rate and diffusion constant the model only recapitulated the behavior over short time scales before the experimental data showed concentration profiles that would not naturally arise from this model. And although this disagreement between data and the model further supports the unlikeliness that dispersion of microtubules is heavily driven by passive diffusion in the network, it also offers some thoughts as to how the model can be refined.

In many models that describe FRAP results and are used to extract key parameters such as binding rates or diffusion rates from experimental data, the fluorescent and photobleached particles are assumed not to interact with each other beyond their Brownian collisions which are treated no differently than their collisions with the surrounding medium. They may undergo chemical reaction and convert to another species, but oftentimes the implementation of FRAP is employed to study a limited set of extensions of the diffusion equation, including diffusion alone [31, 32], reaction-diffusion [33], convectiondiffusion [34], and transport-diffusion [23]. This assumption means that the time evolution of the distribution of fluorescent molecules is mathematically independent of its photobleached counterparts in the quantitative model. In contrast, because the microtubules are crosslinked by motors, their interactions with each other must be taken into account. This feature of the model also suggests that when trying to quantitatively recapitulate the photobleach data, the photobleached microtubule density must also be factored into the equation. This finding further suggests that rather than assuming a constant diffusion D, one may need to model the diffusion as a function of concentration D(c) in Eq. 3.1. And although we coarse-grained the effects of the motors with a linear advection profile as motivated by the unit cell centroid movement, a natural consideration would be to include time-evolving motor densities and creating a system of coupled time-evolving equations [15, 17, 35]. Such an endeavor would suggest the need to photobleach fluorescent motors to examine their dynamics within the network.

Our use of photobleaching to better understand the bulk reorganization of the network during contraction revealed that microtubules are constantly moving relative to one another and interacting with new partners. While our work has provided deeper insights into the extent of filament redistribution during contraction, much is still not known about the origins of the network formation from the initially randomly oriented, uniformly distributed arrangement of filaments prior to contraction. Specifically, the critical states of the formed network that drives the contraction process is still unclear. Photobleaching as applied in the work presented here may prove useful in examining the extent of filament reorganization early on to better understand what sets the conditions for the network to begin to contract. Our findings also offer motivation for examining redistribution of molecules in other actively driven contexts, such as in systems of opposing motors or subject to more complex iLid-micro activation geometries [13, 36].

#### 3.4.1 Materials and methods

#### Microscopy set-up.

The microscopy elements used to activate the iLid-micro dimerization and image the different fluorescence channels are similar to those found in Ross *et al.* [13]. Briefly, a digital light processing projector from Texas Instruments was used to activate the motor dimerization and image the microtubule channels. An excitation filter wheel was placed in front of the projector to filter out the different channels. Photobleaching was performed using a diode laser with a center wavelength of 645 nm. A piezoelectric mirror gimbal mount from Thorlabs was placed downstream of the laser to deflect the beam path over a small range before the laser light passes through a cylindrical lens array inserted into a direct-drive rotation mount. The gimbal mount can then sweep the projected lines laterally to thicken the photobleaching lines before the rotation mount is rotated 90° and the gimbal mount changes the deflecting angle of the beampath in the orthogonal direction. Imaging is performed using a 20x objective. More details are available in the Sec. B.1.6 of Appendix B.

#### Microtubule network assay

The microtubule network formation and contraction assay is set up similarly as in Ross *et al.* [13]. Micro- and iLid-tagged motors are mixed in equal motor monomer ratios with GMPCPP-stabilized microtubules labeled with Alexa 647 in a reaction mix containing among other components ATP, ATP recycling reagents, and crowding agents. While elements of the oxygen scavenging are kept in the reaction, the glucose oxidase and catalase are removed from the reaction to ensure photobleaching. Removal of these oxygen scavengers minimally affects fluorescence intensity during imaging from using the projector, as shown in Sec. B.3 of Appendix B. In cases of examining the photobleached microtubule field while also knowing the density of the entire microtubule field, Alexa 647-labeled microtubules and Alexa 488-labeled microtubules are mixed in ratios that provide roughly the same signal and used in the reaction.

#### Image acquisition arrangement

Control of the light-dimerizing activation, photobleach laser activation, and imaging are performed through the Micro-Manager (MM) software [37, 38] while photobleaching is synchronized using a series of in-house compiled executable files that control the movement of the gimbal and rotation mounts. During acquisition, a customized Beanshell script in MM changes the projection pattern on the DLP to create a circular light pattern for the iLid activation and full field for the imaging channels. When the desired state of the microtubule network is reached for performing photobleaching, the script completes the image acquisition cycle before turning on the photobleaching laser and calling to the executables to create the grid before the next acquisition cycle.

#### Motor purification

Kinesin motors are expressed using the pBiex-1 vector transfected in Sf9 suspension cells. Cells are transfected at 5-7 µg for every  $15 \times 10^6$  cells at a starting concentration  $10^6$  cells per mL of Sf900-III media using a liposome-based transfection regent (Escort IV Transfection Reagent). Cells are harvested  $\sim 60-72$  hours after transfection and purified using the FLAG affinity tag and anti-FLAG antibody resins. Proteins are stored in 50% glycerol by volume with 1.5 mM DTT, 50 µM EDTA, 50 µM EGTA, and 15 µM ATP and stored at - 20°C. Full storage buffers and final concentrations of components are available in Sec. B.1.1 of Appendix B.

### BIBLIOGRAPHY

- T. Vicsek et al., "Novel type of phase transition in a system of self-driven particles", Phys Rev Lett 75 (1995), 1226.
- [2] J. Toner and Y. Tu, "Long-range order in a two-dimensional dynamical XY model: How birds fly together", Phys Rev Lett 75 (1995), 4326.
- [3] S. Ramaswamy, "The mechanics and statistics of active matter", Annu Rev Condens Matter Phys 1 (2010), 323.
- [4] J. Denk and E. Frey, "Pattern-induced local symmetry breaking in activematter systems", Proc Natl Acad Sci 117 (2020), 31623.
- [5] N. Kumar et al., "Flocking at a distance in active granular matter", Nat Commun 5 (2014), 4688.
- [6] A. Kudrolli et al., "Swarming and swirling in self-propelled polar granular rods", Phys Rev Lett 100 (2008), 058001.
- [7] Y. Maroudas-Sacks et al., "Topological defects in the nematic order of actin fibres as organization centres of *Hydra* morphogenesis", Nat Phys 17 (2021), 251.
- [8] K. Copenhagen et al., "Topological defects promote layer formation in Myxococcus xanthus colonies", Nat Phys 17 (2021), 211.
- [9] D. Needleman and Z. Dogic, "Active matter at the interface between materials science and cell biology", Nat Rev Mater 2 (2017), 1.
- [10] F. J. Nédélec et al., "Self-organization of microtubules and motors", Nature 389 (1997), 305.
- [11] T. Surrey et al., "Physical properties determining self-organization of motors and microtubules", Science 292 (2001), 1167.
- [12] M. Schuppler et al., "Boundaries steer the contraction of active gels", Nat Commun 7 (2016), 1.
- [13] T. D. Ross et al., "Controlling organization and forces in active matter through optically defined boundaries", Nature 572 (2019), 224.
- [14] R. Zhang et al., "Spatiotemporal control of liquid crystal structure and dynamics through active patterning", Nat Mater 20 (2021), 875.
- [15] H. Y. Lee and M. Kardar, "Macroscopic equations for pattern formation in mixtures of microtubules and molecular motors", Phys Rev E 64 (2001), 056113.
- [16] F. Nédélec, T. Surrey, and A. Maggs, "Dynamic concentration of motors in microtubule arrays", Phys Rev Lett 86 (2001), 3192.

- [17] S. Sankararaman and G. I. Menon, "Self-organized pattern formation in motor-microtubule mixtures", Phys Rev E 70 (2004), 031905.
- [18] J. M. Belmonte, M. Leptin, and F. Nédélec, "A theory that predicts behaviors of disordered cytoskeletal networks", Mol Syst Biol 13 (2017), 1.
- [19] P. J. Foster et al., "Active contraction of microtubule networks", eLife 4 (2015), 1.
- [20] J. Lippincott-Schwartz, E. Snapp, and A. Kenworthy, "Studying protein dynamics in living cells", Nat Rev Mol Cel Biol 2 (2001), 444.
- [21] J. Braga, J. G. McNally, and M. Carmo-Fonseca, "A reaction-diffusion model to study RNA motion by quantitative fluorescence recovery after photobleaching", Biophys J 92 (2007), 2694.
- [22] "Mobility of cytoplasmic, membrane, and DNA-binding proteins in *Es-cherichia* coli", ().
- [23] M.-V. Ciocanel et al., "Analysis of active transport by fluorescence recovery after photobleaching", Biophys J 112 (2017), 1714.
- [24] J. Lippincott-Schwartz, E. L. Snapp, and R. D. Phair, "The development and enhancement of FRAP as a key tool for investigating protein dynamics", Biophys J 115 (2018), 1146.
- [25] D. Axelrod et al., "Mobility measurement by analysis of fluorescence photobleaching recovery kinetics", Biophys J 16 (1976), 1055.
- [26] S. Fürthauer et al., "Self-straining of actively crosslinked microtubule networks", Nat Phys 15 (2019), 1295.
- [27] A. M. Tayar, M. F. Hagan, and Z. Dogic, "Active liquid crystals powered by force-sensing DNA-motor clusters", Proc Natl Acad Sci 118 (2021), e210873118.
- [28] L. M. Lemma et al., "Multiscale microtubule dynamics in active nematics", Phys Rev Lett 127 (2021), 148001.
- [29] G. Guntas et al., "Engineering an improved light-induced dimer (iLID) for controlling the localization and activity of signaling proteins", Proc Natl Acad Sci 112 (2015), 112.
- [30] N. F. Endres et al., "A lever-arm rotation drives motility of the minusend-directed kinesin Ncd", Nature 439 (2006), 875.
- [31] N. B. Cole et al., "Diffusional mobility of Golgi proteins in membrances of living cells", Science 273 (1996), 797.
- [32] R. Swaminathan, C. P. Hoang, and A. S. Verkman, "Photobleaching recovery and anisotropy decay of green fluorescent protein GFP-S65T in solution and cells: Cytoplasmic viscosity probed by green fluorescent protein translational and rotational diffusion", Biophys J 72 (1997), 1900.

- [33] M. Kang et al., "A quantitative approach to analyze binding diffusion kinetics by confocal FRAP", Biophys J 99 (2010), 2737.
- [34] K. D. Sullivan et al., "Improved model of fluorescence recovery expands the application of multiphoton fluorescence recovery after photobleaching *in vivo*", Biophys J 96 (2009), 5082.
- [35] K. Husain and M. Rao, "Emergent structures in an active polar fluid: Dynamics of shape, scattering, and merger", Phys Rev Lett 118 (2017), 078104.
- [36] Z. Qu et al., "Persistent fluid flows defined by active matter boundaries", Comms Phys 4 (2021), 1.
- [37] A. D. Edelstein et al., "Computer control of microscopes using µManager", Curr Protoc Mol Biol (2010), 14.20.1.
- [38] A. D. Edelstein et al., "Advanced methods of microscope control using µManager software", J Biol Methods 1 (2014), e11.